EFFECT OF ULTRASOUND ON SURVIVAL OF GRAM-NEGATIVE BACTERIA ON CHICKEN SKIN SURFACE

MONIKA KORDOWSKA-WIATER AND DARIUSZ M. STASIAK

Department of Biotechnology, Human Nutrition and Science of Food Commodities,
Department of Meat Technology and Quality Management,
University of Life Sciences in Lublin, 20-704 Lublin, Poland
monika.kordowska-wiater@up.lublin.pl
dariusz.stasiak@up.lublin.pl

Received: November 2, 2010 Accepted: April 21, 2011

Abstract

The objective of the study was to investigate elimination of selected Gram-negative bacteria (Salmonella enterica ssp. enterica sv. Anatum, Escherichia coli, Proteus sp., and Pseudomonas fluorescens) from the surface of chicken wing skin after ultrasound treatment in water and in 1% aqueous lactic acid solution. Samples were intentionally infected with the bacterial strains and then treated with ultrasound at a frequency of 40 kHz and an intensity of 2.5 W/cm² for 3 or 6 min. The degree of reduction of bacteria depended on the duration of ultrasound treatment and the kind of liquid. Sonication in water for 3 min resulted in a reduction of the number of microorganisms on the skin surface to 1.0 log CFU/cm² but longer treatment (6 min) resulted in a reduction of over 1.0 log CFU/cm². The most sensitive was E. coli. Sonication in the lactic acid aqueous solution for 3 min reduced the number of the bacteria by more than 1 log CFU/cm² and after 6 min, the reduction exceeded 1.5 log CFU/cm² (up to 4.0 log CFU/cm²). The bacterium most sensitive to sonication in lactic acid was Pseudomonas. Ultrasound treatment in combination with a lactic acid can be a suitable method for decontamination of poultry carcass skin.

Key words: poultry meat, Gram-negative bacteria, decontamination, ultrasound.

Poultry carcasses are contaminated by microorganisms, which come from skin surface, feathers, intestinal tract, water used for carcass processing, and technological lines. It is assumed that during correct and hygienic slaughter, the total number of microorganisms on the skin surface should not exceed 10⁷ CFU/cm² and the number of proteolytic bacteria should not be more than 8 x 10⁴ CFU/cm² (2, 19). According to the Regulation of the Polish Minister of Agriculture and Rural Development on veterinary requirements for poultry production, the count of coliforms should not exceed 7 x 10⁴ CFU/cm² (Dz.U. 2004.156.1636). Among many microorganisms living on carcasses, two groups play a major role from the point of view of food processing and manufacture. Bacteria from the Enterobacteriaceae family belong to the first group and constitute 10% of the carcass bacterial population and are among the most important bacterial human pathogens (20). Salmonella is the main cause of epidemic infections. Some strains of E. coli, which in healthy humans is a natural constituent of intestinal microflora and is used as an indicator of faecal contamination, can be a serious cause of intestinal disorders. Another bacterium from this family is Proteus sp., which has decay ability. Proteolytic and lipolytic microorganisms belong to the second group. Rods of Pseudomonas and Moraxella–Acinetobacter, which are dominant in this group of bacteria, constitute about 50% of the total microbial population after slaughter and are the main group of bacteria during cold storage (20).

To ensure a suitable technological and trade quality and shelf-life of carcasses, investigations has been carried out into bacterial elimination using sonication by ultrasound with special parameters (4, 10, 11, 13). Ultrasound can be used to inactivate bacteria and deaggregate bacterial clusters or flocks through a number of physical, mechanical, and chemical effects arising from acoustic cavitation. On collapse, cavitation bubbles produce enough energy to mechanically weaken or disrupt bacteria via a number of processes (8, 9, 14). The mechanism of microbial killing is mainly due to thinning of cell membranes, localized heating, and production of free radicals. During sonication, longitudinal waves are created when a sonic wave meets a liquid medium, thereby creating regions of alternating compression and expansion (13). The effect of bacterial inactivation depends on action time, intensity, frequency sensitivity of cells, kind of sonication environment, and presence of additional substances (3). In different investigations, a reduction of microbiological contamination by 2 or more logarithms was obtained (11, 13, 17).

The purpose of the presented study was to investigate the reduction of selected Gram-negative bacteria on the surface of poultry skin (wings) as an effect of treatment with low-frequency and medium-intensity ultrasounds in water or a lactic acid aqueous solution in working aliquot.
Material and Methods

The chicken wings were obtained directly from the technological line of a local poultry slaughterhouse. Gram-negative bacteria: Salmonella enterica ssp. enterica sv. Anatum, Escherichia coli, Proteus sp., and Pseudomonas fluorescens obtained from the collection of the Department of Biotechnology, Human Nutrition and Science of Food Commodities of the University of Life Science in Lublin. The bacteria were incubated in a nutrient broth for 24 h at 37°C (Pseudomonas at 30°C), and contamination of the wings was done by plunging them in bacterial suspensions, separate for each strain, for 1 min. The infected wings were packed in a sterilised aluminum foil and kept for 30 min at room temperature to enable better adsorption of the bacteria to the skin.

Sonication was carried out in a bath device (Polsonic, Poland) at a frequency of 40 kHz and an intensity of 2.5 W/cm² in sterile distilled water or in sterile 1% solution of aqueous lactic acid at 20°C. In experiment A, the wings were put into a chamber to be totally submerged in working solution and sonication was continued for 3 and 6 min. Each sample was sonicated after an exchange of the solution. In experiment B, the wings were sonicated without exchanging the solution of lactic acid. Sonication of every wing contaminated with S. Anatum and E. coli was continued for 6 min, whereas the treatment time for every wing inoculated with Proteus and Pseudomonas was 3 min. Three successive series were run, immediately following one another, so it means that working aliquot used in this experiment was under continuous ultrasound action for 18 min (3 x 6 min) and 9 min (3 x 3 min), respectively. Controls (unsonicated samples of wings) were used in both experiments. After sonication time, the samples were withdrawn, and then two smears from 4 cm² of surface were taken from each wing for a microbiological analysis. Smears were also collected from control wings. Decimal dilutions were prepared from each smear to determine the presence of living bacteria on the skin. One cubic centimetre of solution was taken from the sonic bath after each sonication to prepare dilutions and to determine the presence of alive bacteria in the working solution.

Microbiological analysis. One cubic centimetre of each dilution was spread on sterile Petri dishes. BGA, VRBL, Nogrady, and King B agar (BTL, Poland) were used for growth of S. Anatum, E. coli, Proteus sp., and P. fluorescens, respectively. Colonies of Salmonella were counted after 24-h incubation at 37°C, whereas colonies of E. coli and Proteus were counted after 48-h incubation at the same temperature. P. fluorescens was incubated at 30°C for 72 h and then counted. The numbers of colony forming units (CFU) in 1 cm² of solution or in 1 cm² of chicken wing surface are presented as logarithms of CFU. The results are presented as means of two independent experiments, with a standard deviation.

Results

Bacterial suspensions used for wing contamination contained different average numbers of living cells as follows: S. Anatum 8.12 ± 1.59 log CFU/cm², E. coli 7.86 ± 1.53 log CFU/cm², Proteus sp. 5.57 ± 0.99 log CFU/cm² and P. fluorescens 6.42 ± 0.37 log CFU/cm². The numbers of bacteria observed on surface of contaminated wings are presented in Table 1.

The degree of reduction of bacterial counts depended on sonication time and the application of the lactic acid aqueous solution as working solution (Fig. 1) (experiment A). Sonication continued in water for 3 min reduced the number of microorganisms below or about 1 log CFU/cm², but 6 min were generally more effective. The strain, which was most sensitive to the action of ultrasound in water was E. coli.

![Graph](image)

Fig. 1. The degree of reduction of bacterial cells on the skin surface of wings after sonication in water (AD) or aqueous solution of lactic acid (LA).

After 3 min of sonication in the lactic acid solution, the reduction in bacterial cell counts exceeded 1 log (with the exception of E. coli), and for P. fluorescens it reached about 3 log CFU/cm². After 6 min of ultrasounds action, the reduction effect ranged from 1.39 to 4 logarithmic cycles. The bacterium most sensitive to the synergistic effect of lactic acid and sonication was Pseudomonas strain and the most resistant bacterium was E. coli (Fig 1 and Table 1). The increase in the solution temperature in the ultrasonic device during sonication, connected with dissipation, was about 2°C after 3 min and about 4°C after 6 min of work.

The experiment concerning the presence of bacteria in the sonic environment showed that they were able to survive in quite high numbers in water (Table 1). E. coli survived in 1% solution of lactic acid, but extension of the sonication time from 3 to 6 min reduced their number from 2 to 1 log CFU/cm². The other bacteria were not detectable in the lactic acid solution.

To evaluate the technological potential of the application of this method, the influence of ultrasounds on the examined Gram-negative bacteria in non-exchangeable 1% solution of lactic acid (experiment B)
was investigated. The more resistant bacteria were sonicated for 6 min (S. Anatum and *E. coli*) and the more sensitive bacteria were treated for 3 min. Three successive series were run, immediately following one another. It was shown that the lethal effect was continued for all the series (Table 2). The number of *Salmonella* decreased by about 1.3-2.0 log CFU/cm² and no alive bacteria were detected in the working solution. A decrease in *E. coli* rods on the wing surfaces was in the order of 1.06-1.7 log CFU/cm², but they survived in the working solution. Three-minute sonication of *Proteus* sp. and *P. fluorescens* in the lactic acid solution effectively decreased the number of these bacteria by 0.88-1.35 log CFU/cm² and by above 3 log CFU/cm² with total elimination of living cells from the working solution.

**Discussion**

Immersion sonication by ultrasound of low frequency and medium intensity reduced the number of Gram-negative bacteria on the surface of chicken skin. The hydrodynamic effects connected with changes in the acoustic pressure and cavitation are the cause of the reduction of microorganisms on the skin. A transition of bacteria from food surface into the working solution was detected. Gram-negative microorganisms, having an outer membrane, are usually susceptible to limited sonication. Gradients of pressure caused by ultrasound are the cause of disturbances in diffusion across the cell membrane.

They enable ions to penetrate into the cytoplasm, which disturbs cell metabolism and other vegetative processes possibly leading to death. This effect is enhanced by free radicals obtained in sonochemical reactions (6, 8, 13). For that reason the level of reduction of the number of Gram-negative strains of *S. Anatum, E. coli, Proteus* sp., and *P. fluorescens* was higher in the aqueous solution of lactic acid than in water. Among the examined organisms, the strain of *E. coli* was the most sensitive to ultrasound in water (a reduction of 2.27 log CFU/cm²).

**Table 1**

Number of bacteria on skin surface (log CFU/cm²) and in working solution (distilled water, 1% solution of lactic acid) (log CFU/cm²) in experiment A (± standard deviation)

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Environment</th>
<th>Distilled water</th>
<th>Lactic acid solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>3 min</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>skin</td>
<td>3.36±0.44</td>
<td>2.7±0.29</td>
</tr>
<tr>
<td>Anatum</td>
<td>solution</td>
<td>0.00±0.00</td>
<td>5.22±0.06</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>skin</td>
<td>4.16±0.38</td>
<td>3.09±0.76</td>
</tr>
<tr>
<td></td>
<td>solution</td>
<td>0.00±0.00</td>
<td>4.66±0.35</td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>skin</td>
<td>3.6±0.89</td>
<td>2.88±0.76</td>
</tr>
<tr>
<td></td>
<td>solution</td>
<td>0.00±0.00</td>
<td>2.05±1.4</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>skin</td>
<td>4.32±0.16</td>
<td>3.69±0.14</td>
</tr>
<tr>
<td><em>fluorescens</em></td>
<td>solution</td>
<td>0.00±0.00</td>
<td>3.79±0.22</td>
</tr>
</tbody>
</table>

**Table 2**

Number of bacteria on skin surface (log CFU/cm²) and cumulated number of bacteria in 1% solution of lactic acid (LA) (log CFU/cm²) in experiment B (± standard deviation)

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Environment</th>
<th>Sonication time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>skin</td>
<td>3.38±0.5</td>
</tr>
<tr>
<td>Anatum</td>
<td>LA</td>
<td>0.00±0.0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>skin</td>
<td>3.53±0.46</td>
</tr>
<tr>
<td></td>
<td>LA</td>
<td>0.00±0.0</td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>skin</td>
<td>4.27±0.47</td>
</tr>
<tr>
<td></td>
<td>LA</td>
<td>0.00±0.0</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>skin</td>
<td>3.89±0.61</td>
</tr>
<tr>
<td><em>fluorescens</em></td>
<td>LA</td>
<td>0.00±0.0</td>
</tr>
</tbody>
</table>
The microorganism most sensitive to the synergistic effect of ultrasound and lactic acid was *Pseudomonas* (a reduction of 4.1 log CFU/cm³). The sonication time was an important agent in elimination of bacteria from the wing skin. Effective reduction of bacterial numbers on the skin surface was observed both after sonication in microbiologically clean working solution and in the one used in previous sonication of infectious material. Cells of *Salmonella*, *Proteus*, and *Pseudomonas* were also absent in working aliquot after three series of ultrasound treatment. Villamiel and Jong (18) investigated the influence of ultrasound at a frequency of 20 kHz on *P. fluorescens* at a higher temperature. During continuous sonication in TSB broth for 22.5 and 34 min, a reduction of *Pseudomonas* of 0.6 ±0.1 and 0.8 ±0.0 log CFU/cm³, respectively, was observed. A considerable extension of the sonication time to 56 min resulted in a 3 log CFU/cm³ reduction in cell numbers but simultaneously the temperature of the environment increased to 61°C (18).

Inactivation of *Pseudomonas aeruginosa* depended on sonication time and the intensity of ultrasounds (5, 15). An almost total inactivation of these bacteria can be obtained by the use of ultrasounds at a frequency of 26 kHz, especially at a medium of pH 4. After sonication of broiler skin surface by ultrasound at a frequency of 47 kHz during a time range of 0.5-30 min, *Salmonella* cells survived probably due to the protective action of the irregular surface of the skin (15). Sonication of carcasses in a 0.5 ppm chlorine solution showed a synergism of effects (10, 11). The best reduction of the number of bacteria was obtained as a synergistic effect arising from the interaction of sonication time and temperature, the chemical composition, pressure, and viscosity of the medium (1, 3, 6, 7, 12, 16). In water the cells were mostly washed off rather than destroyed by ultrasounds, and the effectiveness of this process depended on the coarseness of the sonicated surface. The reaction environment of lactic acid in combination with ultrasounds acted bactericidally on almost all of the examined Gram-negative bacteria.

6. Huang E., Mittal G.S., Griffiths M.W.: Inactivation of *Salmonella enteritidis* in liquid whole egg using combination treatments of pulsed electric field, high pressure and ultrasound. Biosystems Engin 2006, 94, 403-413.